TITLE OF THE INVENTION

[0001] OPTICAL SENSORS BASED ON HYBRID APTAMER/CONJUGATED POLYMER COMPLEXES

FIELD OF THE INVENTION

[0002] The present invention relates to optical sensors. More specifically, the present invention is concerned with optical sensors based on hybrid aptamer/conjugated polymer complexes.

BACKGROUND OF THE INVENTION

[0003] Intense research is being carried out worldwide with the goal of developing rapid, simple, specific, and sensitive detection tools for medical diagnostics and biomedical research applications. Fundamentally, most analytical tests and immunoassays rely on molecular recognition and its transduction into a measurable output. Among all the possible molecular recognition elements, artificial nucleic acid ligands (aptamers) have recently attracted a lot of interest due to their capability of binding various metal ions, amino acids, drugs, proteins, as well as other molecules having high affinity and specificity.[1-11]

[0004] Aptamers are usually isolated from combinatorial libraries of synthetic nucleic acids by an iterative process of adsorption, recovery, and amplification coined as SELEX (*Systematic Evolution of Ligands by Exponential Procedure*). Aptamer-based ligands constitute highly promising candidates for the specific detection of various molecules. Additionally, they can also be used in competition binding assays, such as for example in high-throughput screening assays[7], for the identification of new potential drugs

capable of displacing the aptamers from their targets.

[0005] The above-mentioned approaches, however, require adequate transducing (*i.e.* reporting) elements in order to generate a physically measurable signal resulting from the recognition event. Binding of an aptamer to a target protein, for example, has been detected by using fluorescence (*e.g.* molecular beacons[12-13]) or by using a quartz microbalance[14]. In most cases, however, these methods either involve a tagging process or sophisticated experimental techniques. Furthermore, it is worth noting that labeling with various functional groups may even compromise the binding properties of the aptamers.

[0006] There thus remains a need to develop a rapid, simple, specific and sensitive detection tool capable of transducing the binding of an aptamer to its target into a clear signal.

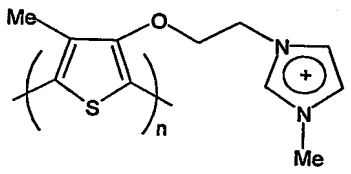
[0007] The present invention seeks to meet these and other needs.

[0008] The present invention refers to a number of documents, the content of which is herein incorporated by reference in their entirety.

SUMMARY OF THE INVENTION

[0009] The present invention relates to optical sensors based on hybrid aptamer/conjugated polymer complexes. More specifically, the present invention relates to the use of a water-soluble cationic polythiophene derivative as a "polymeric stain" capable of specifically transducing the binding of an aptamer to its target into a clear optical (colorimetric or fluorometric) signal.

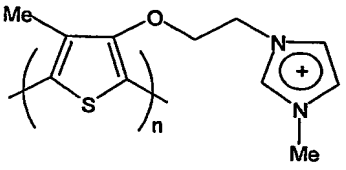
[0010] The present invention relates to an optical sensor for detecting a target comprising a single-stranded aptamer complementary to the target, and a water-soluble cationic polythiophene derivative of the following formula:



wherein "n" is an integer ranging from 6 to 100.

[0011] The present invention also relates to a method for detecting a target comprising the steps of:

a) contacting a sample suspected of containing the target with an optical sensor, the optical sensor including a single-stranded aptamer complementary to the target, and a water-soluble cationic polythiophene derivative of the following formula:



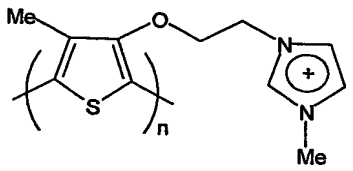
wherein "n" is an integer ranging from 6 to 100; and

b) detecting binding of the aptamer to the target by measuring an optical signal.

[0012] In addition, the present invention also relates to a method for detecting a target comprising the steps of:

a) contacting a sample suspected of containing the target with an aptamer known to be complementary to the target;

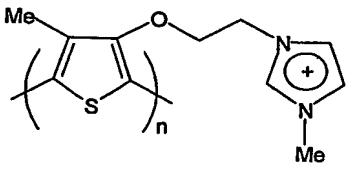
b) further contacting the sample with a water-soluble cationic polythiophene derivative of formula:



wherein "n" is an integer ranging from 6 to 100; and

c) detecting binding of the aptamer to the target by measuring an optical signal.

[0013] Furthermore, the present invention also relates to the use of an optical sensor comprising a single-stranded aptamer and a water-soluble cationic polythiophene derivative of the following formula:



wherein "n" is an integer ranging from 6 to 100, for detecting a target, the aptamer being complementary to the target.

[0014] In a particular embodiment of the present invention, the target is human α-thrombin.

[0015] In a further particular embodiment of the present invention, the target is D-adenosine.

[0016] Further scope and applicability will become apparent from the detailed description given hereinafter. It should be understood however, that this detailed description, while indicating preferred embodiments of the invention, is given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] Having thus generally described the invention, reference will now be made to the accompanying drawings, showing by way of illustration preferred embodiments thereof, and in which:

[0018] Figure 1A shows photographs of polymer **1**: (a) alone; (b) in the presence of X1 in LiCl 0.01M; (c) in the presence of X1 in NaCl 0.01M; (d) in the presence of X1 in KCl 0.01M; and (e) in the presence of X1 in RbCl 0.01M; Figure 1B shows the UV-Vis absorption spectra of polymer **1** (2.9×10^{-9} mole on a monomer unit basis) in the presence of X1 (1.9×10^{-10} mole of the 15-mer) and different salts in 100 μL of water at 25°C;

[0019] Figure 2 shows the complexation between unfolded anionic ss-DNA and polymer **1** (Path A), as well as the complexation between unfolded anionic ss-DNA and polymer **1** in the presence of potassium ions (Path B);

[0020] Figure 3 illustrates the specific detection of human α-thrombin using ss-DNA thrombin aptamer X1 and positively-charged polymer **1**;

[0021] Figure 4 illustrates the UV-Visible absorption spectrum of: (a) polymer **1** in water at 25°C; (b) a complex (1/1/1) human thrombin/X1/polymer

1, in water at 25°C (c) a mixture (1/1/1) of human thrombin/X2/polymer **1**, in water at 25°C; and (d) a mixture (1/1/1) of BSA/X1/ polymer **1**, in water at 25°C;

[0022] Figure 5 illustrates the fluorescence spectrum measured at 5°C of: (a) polymer **1**; (b) human thrombin/X1/polymer **1** complex (1/1/1); (c) human thrombin/X2/polymer **1** mixture (1/1/1); and (d) X1/polymer **1** complex (1/1) in water;

[0023] Figure 6 illustrates the UV-visible spectrum of: (a) 1.08×10^{-7} mol of cationic polymer **1** in 200 µl of water at 5°C; (b) a mixture of 2.9×10^{-9} mol of D-adenosine, 1.08×10^{-7} mol of DNA D-adenosine aptamer (based on monomeric negative charge or 2.9×10^{-9} mol of 37-mers) and 1.08×10^{-7} (based on charge unit) of polymer **1** in 200 µl of water at 5°C; and (c) a mixture of 2.9×10^{-9} mol of L-adenosine, 1.08×10^{-7} mol of DNA D-adenosine aptamer (based on monomeric negative charge or 2.9×10^{-9} mol of 37-mers) and 1.08×10^{-7} (based on charge unit) of polymer **1** in 200 µl of water at 5°C; and

[0024] Figure 7 illustrates the emission spectrum of: (a) 1.08×10^{-7} mol of cationic polymer **1** in 200 µl of water at 5°C; (b) a mixture of 2.9×10^{-9} mol of D-adenosine, 1.08×10^{-7} mol of DNA D-adenosine aptamer (based on monomeric negative charge or 2.9×10^{-9} mol of 37-mers) and 1.08×10^{-7} (based on charge unit) of polymer **1** in 200 µl of water at 5°C; (c) a mixture of 2.9×10^{-9} mol of L-adenosine, 1.08×10^{-7} mol of DNA D-adenosine aptamer (based on monomeric negative charge or 2.9×10^{-9} mol of 37-mers) and 1.08×10^{-7} (based on charge unit) of polymer **1** in 200 µl of water at 5°C.

[0025] Other objects, advantages and features of the present invention will become more apparent upon reading of the following non-

restrictive description of preferred embodiments with reference to the accompanying drawings which is exemplary and should not be interpreted as limiting the scope of the present invention.

DETAILED DESCRIPTION OF SPECIFIC EMBODIMENTS

[0026] Unless defined otherwise, the scientific and technological terms and nomenclature used herein have the same meaning as commonly understood by a person of ordinary skill. Nevertheless, definitions of selected terms are provided for clarity and consistency.

[0027] As used herein, the term "optical sensor" is understood as referring to a complex consisting of a single-stranded aptamer and a water-soluble, cationic polythiophene derivative, the aptamer being complementary to a target to be detected, allowing for the detection of the target via optically measurable means. Without being so limited, these means include UV-visible or fluorescence spectra.

[0028] As used herein, the term "aptamer" is understood as being a single-stranded oligonucleotide that binds to a specific molecular target, non limiting examples of which are potassium ions, small organic molecules, amino acids, proteins, whole cells and nucleotides.

[0029] As used herein, when referring to an aptamer, the term "complementary" is understood as referring to the ability of the aptamer to hybridize with a specific chemical or biochemical target.

[0030] As used herein, the expression "enantiomeric resolution" is

understood as referring to a discrimination (identification) between two enantiomers.

[0031] As used herein, the expression "folded structure" is understood as referring to a non-linear conformational structure.

[0032] As used herein, the term "target" is understood as referring to a charged entity, non-limiting examples of which are potassium ions, small organic molecules, amino acids, proteins, whole cells and nucleotides.

[0033] In a broad sense, the present invention relates to optical sensors based on hybrid aptamer/conjugated polymer complexes. More specifically, the present invention relates to the use of a water-soluble cationic polythiophene as a "polymeric stain" capable of specifically transducing the binding of an aptamer to its target into a clear optical (colorimetric or fluorometric) signal. The optical sensors do not require any chemical reaction to take place on the probes or with the analytes. Instead, the use of the optical sensors of the present invention is based on conformational changes as well as on electrostatic interactions between a cationic polythiophene derivative (*i.e.* poly(3-alkoxy-4-methylthiophene), an anionic single-stranded oligonucleotide (aptamer) and a target to be detected.

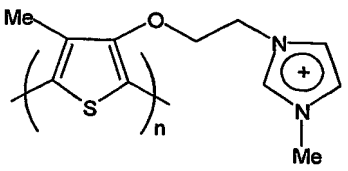
[0034] The present invention also relates to methods for detecting a charged entity using such optical sensors. Non-limiting examples of such charged entities include potassium ions, small organic molecules, amino acids, proteins, whole cells and nucleotides.

[0035] The use of the optical sensors of the present invention, for instance, allows to specifically detect as few as 2×10^{-15} mol of human

thrombin or 2 x 10^{-14} mol of D-adenosine in only a few minutes and could be easily adapted for use in detecting many other chemical or biochemical targets non-limiting examples of which are ions, small organic molecules, amino acids, proteins, whole cells and nucleotides. Indeed, it is well known that the optical, electrical and electrochemical properties of polythiophenes are essentially constant after a minimum of 6-8 repeating units (P. Bäuerle, *The synthesis of Oligothiophenes*, Chapter 3 in Handbook of Oligo- and Polythiophenes, D. Fichou Ed., Wiley-VCH, Weinheim, pp. 89-181, 1999). A person skilled in the art would therefore understand that any polymer **1** having from 6 to 100 repeating units would work within an optical sensor as contemplated by the present invention. Furthermore, it is well known that aptamers with high affinity and selectivity have been created against a variety of targets, such as small organic molecules, peptides, proteins, and even cells (M. Famulok, G. Mayer, M Blind, *Acc. Chem. Res.* **33**, 591-599, 2000). It is to be understood that any aptamer complementary to a target to be detected is within the scope of the present invention.

[0036] Single-stranded DNA (aptamer) can specifically bind potassium ions, human α-thrombin, or D-adenosine for instance. When binding takes place, the aptamer undergoes a conformational transition from an unfolded to a folded structure. This conformational change of the negatively-charged oligonucleotide can be detected by adding a water-soluble, cationic polythiophene derivative which transduces the new complex formation into an optical (colorimetric or fluorometric) signal without any labeling of the probe or of the target.

[0037] In a particular embodiment, an optical sensor according to the present invention comprises a polythiophene derivative, referred to herein as polymer **1**, having the following formula:



wherein "n" is an integer ranging from 6 to 100 (see Figure 1).

[0038] The cationic, water-soluble, electroactive, and photoactive polymer **1** was prepared according to known literature procedures.[15] As is observed for most poly(3-alkoxy-4-methylthiophene)s,[16-19] polymer **1** exhibits chromic properties (color changes) which are due to conformational changes of the flexible conjugated backbone. Moreover, polymer **1** is known to display important optical changes when complexed to ss-DNA or ds-DNA[15], making it a good candidate for transducing the binding of an aptamer to a given target.

[0039] The monovalent potassium cation is known for its folding-inducing properties in several classes of nucleic acids.[20,21] As shown in Figure 1, an aqueous solution of polymer **1** is yellow with a maximum absorption (λ_{max}) at 402 nm (Figure 1A,a and B,a). This absorption maximum at a relatively short wavelength is related to a random-coil conformation of the polythiophene derivative, as any twisting of the conjugated backbone leads to a decrease of the effective conjugation length.[16]

[0040] A red color (λ_{max}= 527 nm) was observed in the presence of LiCl (Figure 1A,b and B,b); NaCl (Figure 1A,c and B,c) or RbCl (Figure 1A,e and B,e) and ss-DNA (sequence X1: 5'-GGTTGGTGTGGTTGG-3'). This red color shift is associated with a stoichiometric complexation between the unfolded anionic ss-DNA and the cationic polythiophene derivative (Figure 2, path A). Such stoichiometric polyelectrolyte complexes tend to be insoluble in

the medium in which they are formed and appear as aggregates.[15] These red-violet aggregates (probably formed from planar polymer chains) possess an absorption spectrum similar to that obtained in the solid state.

[0041] The optical properties (Figure 1A,d and B,d), however, are different when potassium ions are present. As a result of the formation of a folded structure (quadruplex form) of oligonucleotide X1, stabilized by potassium ions (K^+), polymer **1** is able to wrap itself around this structure through electrostatic interactions (Figure 2, Path B). Similar results were also observed when the chloride counter-ion was replaced by a bromide or iodide counter-anion, indicative of the specificity of the detection towards potassium cations.

[0042] In a particular embodiment of the present invention, human α-thrombin was selected as an example of a target to be detected since X1 ss-DNA sequence (5'-GGTTGGTGTGGTTGG-3') is known to be a specific binding sequence (*i.e.* an aptamer) of this protein. On the other hand, the oligonucleotide ss-DNA (X2: 5'-GGTGGTGGTTGTGGT-3') is known to be a non-binding sequence.[22] A conformational change occurs in the aptamer X1 when it binds to the thrombin molecule. Both NMR and X-ray diffraction studies have revealed that the aptamer adopts a compact unimolecular quadruplex structure with two G-quartets.[23, 24]

[0043] As shown in Figure 3, the specific detection of human α-thrombin is realized due to the formation of a quadruplex structure of the thrombin aptamer (X1). Accordingly, the 1:1:1 complex between polymer **1**, X1, and α-thrombin has a similar orange color and UV-Visible absorption spectrum than that induced by K^+ (Figures 4b and 1B,d). Human α-thrombin promotes the formation of a folded structure (quadruplex form) of thrombin aptamer X1,

enabling cationic polymer **1** to wrap itself around this quadruplex structure, which seems to partially hinder the aggregation and planarization of the polymer **1** when in the presence of ss-DNA X1 (Figure 3, Path A). It is worth noting that only the stoechiometry of the aptamer (in terms of negative charges) and of polymer **1** (in terms of positive charges) has to be balanced, whereas an excess of α-thrombin does not influence its detection.

[0044] The specificity of the detection was verified by two control experiments carried out under identical conditions. In a first control experiment a non-binding sequence ss-DNA (X2: 5'-GGTGGTGGTTGTGGT-3') was used (Figure 4c) and in a second control experiment BSA (bovine serum albumin) was used (Figure 4d). In both cases, an important red-shift toward lower energy (λ_{max}=505 nm) was observed. Furthermore, the color of these solutions was red-violet, which is typical of the planar and highly conjugated structure of the polythiophene backbone when mixed with unfolded ss-DNA (Figure 3, Path B and Figure 2, Path A). The detection limit of this colorimetric method is about $1x10^{-11}$ mole of thrombin in a total volume of *ca.* 100 μL (a concentration of about $1x10^{-7}$ M).

[0045] The fluorescent properties of conjugated polymers can also be utilized to detect very small quantities of analytes.[25-29] The fluorometric detection of the binding of the thrombin aptamer to human α-thrombin is possible because the fluorescence of poly(3-alkoxy-4-methylthiophene) is quenched in the planar, aggregated form.[15-19] The yellow, random-coil form of polymer **1** is fluorescent (Figure 5a) with an emission maximum at 525 nm. When non-specific thrombin aptamer (X2) is used (Figure 5c), or in the absence of human α-thrombin (Figure 5d), the now red-violet, highly conjugated form has a much lower fluorescence intensity and the maximum of emission is red-shifted (λ_{em}=590 nm). However, when the 1:1:1 complex

(human α-thrombin /thrombin aptamer X1/polymer **1**) is formed (Figure 5b), the resulting orange intermediate form is less fluorescent than the yellow form but more fluorescent (*ca.* a six-fold increase) than the red-violet form. This higher intensity of emission could be related to a partially planar conformation of the polythiophene chain, but with less aggregation of the chains.[30]

[0046] The use of a standard spectrofluorimeter provides for a detection limit of 2×10^{-15} mole (a concentration of 1×10^{-11} M in 200 μL) of human α-thrombin.

[0047] The resolution of small molecule enantiomers is of great importance in many research fields such as biochemistry and drug analysis. Two enantiomers of a same molecule may have distinct physiological behaviors; one enantiomeric form can be pharmaceutically active whereas the other enantiomeric form can be inactive or toxic.

[0048] In a further particular embodiment of the present invention, D-adenosine was selected as an example of a target to be detected.

[0049] More specifically, the enantiomeric resolution of D-adenosine and L-adenosine was performed using DNA aptamer (5'-ATTATACCTGGGGGAGTATTGCGGAGGAAGGTATAAT-3') (31).

[0050] In a first step, a framework composed of two stacked G-quartets is assumed by mixing D-adenosine and DNA aptamer (31) (5'-ATTATACCTGGGGGAGTATTGCGGAGGAAGGTATAAT-3'). The formed complex is more stable at 5°C. The cationic polymer **1** is then added and is assumed to wrap itself around the previously formed complex. The

stoichiometry of the adenosine enantiomer/aptamer/polymer **1** complex is 1:1:1.

[0051] A series of identical steps was then performed using L-adenosine. Since L-adenosine is not supposed to induce a conformational change in DNA aptamer (31) (5'-ATTATACCTGGGGGAGTATTGCGGAGGAAGGTATAAT-3'), the cationic polymer **1** should bind to the aptamer and lead to the formation of a duplex.

[0052] The cationic polymer **1** is yellow in aqueous solution (λ = 397 nm) (Figure 6a). Its maximum absorption is related to its random-coil conformation and is indicative of a decrease in the conjugation length. When L-adenosine is put in the presence of the aptamer (31) and polymer **1**, a color change from yellow to red (λ = 397nm to λ = 500nm) (Figure 6c) takes place. This reveals a planarization and aggregation of the polymer backbone through electrostatic interactions and an increase of the conjugation length. A partial return to its yellow form is observed (λ = 410nm with a shoulder at 510nm) (Figure 6b), when a complex is formed between the D-adenosine and aptamer (31), in presence of polymer **1**. The detection limit by UV-visible absorption is about 1.8×10^{-10} mole of D-adenosine in a total volume of ca. 200µL (which gives a concentration of about 9×10^{-7} M).

[0053] Since fluorescence spectroscopy is more sensitive than UV-visible absorption spectroscopy, the emission properties of polymer **1** can thus be used to detect even smaller quantities of D-adenosine. The yellow aqueous solution of polymer **1** is fluorescent with a maximum of emission at 525 nm (Figure 7a). When L-adenosine is put in presence of aptamer 31 and polymer **1**, the fluorescence of the red solution obtained is red-shifted and quenched (Figure 7b). In the case of complexation between D-adenosine and aptamer 31

in presence of polymer **1**, a partial recovery of the fluorescence could be observed (Figure 7c). The detection limit using a standard spectrofluorimeter is about 1.8×10^{-14} mole of D-adenosine in a total volume of ca. 200 µL (which gives a concentration of about 9×10^{-11} M).

EXPERIMENTAL

UV-Visible measurements

[0054] All UV-Visible absorption spectra were taken using a Hewlett-Packard (model 8452A) spectrophotometer.

Fluorescence measurements

[0055] All fluorescence spectra were recorded on a Carry Eclipse (Varian Inc.) spectrofluorimeter. The excitation was performed at 420 nm.

EXAMPLE 1

Detection of cations

[0056] In a quartz cell having an optical path length of 1.0 cm, 4µL [2.9×10^{-9} mole (based on negative charges)] of 15-mer X1 were added to 100µL of an aqueous solution of a given alkali metal cation (10 mM) (chloride salts), followed by the addition of 4µL [2.9×10^{-9} mol (based on positive charge)] of a solution of cationic polymer **1**. All UV-Visible absorption spectra were recorded at room temperature. The results are illustrated in Figure 1.

EXAMPLE 2

Detection of human α-thrombin

[0057] In a UV quartz cell having an optical path length of 1.0 cm, 1.9×10^{-10} mol of human α-thrombin (Haematologic Technologies Inc.) (the initial concentrated solution was diluted with sterilized water to obtain the

appropriate concentration) and 2.9 x 10^{-9} mol (based on negative charges or 1.9x10^{-10} mol of 15-mer) of ss-DNA thrombin aptamer X1 were mixed in 100 µL of pure water at 25°C. This was followed by the addition of 2.9 x 10^{-9} mol (based on charge repeat unit) of polymer **1**, to form a complex (1/1/1).

[0058] Two control experiments were carried out using a non-specific sequence X2 and BSA (bovine serum albumin, obtained from Sigma), under identical conditions. The results are illustrated in Figure 4.

EXAMPLE 3

Detection of D-adenosine

[0059] In a UV quartz cell having an optical path length of 1.0 cm, 2.9 x 10^{-9} mol of D-adenosine and 1.08 x 10^{-7} mol of DNA D-adenosine aptamer (based on monomeric negative charges or 2.9x10^{-9} mol of 37-mers) were mixed in 200 µL of pure water at 5°C. This was followed by the addition of 1.08 x 10^{-7} mol (based on charge repeat unit) of polymer **1**, to form a complex (1/1/1).

[0060] A control experiment, under identical conditions, was carried out using L-adenosine, to which the D-adenosine aptamer is not complementary. The results are illustrated in Figure 6.

EXAMPLE 4

Detection of human α-thrombin

[0061] In a fluorescence cell having an optical path length of 3.0 mm, 3.8 x 10^{-10} mol of human α-thrombin and 5.7 x 10^{-9} mole (based on monomeric negative charge or 3.8 x 10^{-10} mol of 15-mer) of ss-DNA thrombin aptamer X1 were mixed in 200 µl of pure water, followed by the addition of 5.7

x 10^{-9} mol (based on charge repeat unit) of polymer **1**. The fluorescence spectrum of all mixtures was recorded at 5°C. For the lower concentration of human α-thrombin, the excitation was performed at 420 nm, and the fluorescence emission intensity was measured at 584 nm (without recording the entire emission spectrum).

[0062] A control experiment was carried out using a non-specific sequence X2 under identical conditions. The results are illustrated in Figure 5.

EXAMPLE 5

Detection of D-adenosine

[0063] In a fluorescence cell having an optical path length of 3.0 mm, 2.9 x 10^{-9} mol of D-adenosine and 1.08 x 10^{-7} mol of DNA D-adenosine aptamer (based on monomeric negative charge or 2.9 x 10^{-9} mol of 37-mers) were mixed in 200 µl of pure water, followed by the addition of 1.08 x 10^{-7} mol (based on charge repeat unit) of polymer **1**. The fluorescence spectrum of all mixtures was recorded at 5°C. The results are illustrated in Figure 7.

[0064] A control experiment, under identical conditions, was carried out using L-adenosine, to which the D-adenosine aptamer is not complementary. The results are illustrated in Figure 7.

[0065] Although the present invention has been described hereinabove by way of preferred embodiments thereof, it can be modified, without departing from the spirit and nature of the subject invention as defined in the appended claims.

REFERENCES

1. M. P. Robertson, G. F. Joyce, *Nature* **344**, 467 (1990).
2. A .D. Ellington, J. W. Szostak, *Nature* **346**, 818 (1990).
3. C. Tuerk, L. Gold, *Science* **249**, 505 (1990).
4. C. K. O'Sullivan, *Anal. Bioanal. Chem.* **372**, 44 (2002).
5. S.L. Clark, V.T. Remcho, *Electrophoresis* **23**, 1335 (2002).
6. D.W. Drolet, L.M. McDermott, T.S. Romig, *Nature Biotechnol.* **14**, 1021 (1996).
7. L.S. Green, C. Bell, N. Janjic, *Biotechniques* **30**, 1094 (2001).
8. K.A. Davis, B. Abrams, Y. Lin, S.D. Jayasena, *Nucleic Acids Res.* **24**, 702 (1996).
9. Y. Lin, D. Nieulandt, A. Magallanez, B. Feistner, S.D. Jayasena, *Nucleic Acids Res.* **24**, 3407 (1996).
10. M. Blank, T. Weinschenk, M. Priemer, H. Schluesener, *J. Biol. Chem.* **276**, 16464 (2001).
11. M. Lee, D.R. Walt, *Anal. Biochem.* **282**, 142 (2000).
12. N. Hamaguchi, A. Ellington, M. Stanton, *Anal. Biochem.* **294**, 126 (2001).
13. J.L. Jianwei, F. Xiaohong, T. Weihong, *Biochem. Biophys. Res. Commun.* **292**, 31 (2002).
14. M. Liss, B. Petersen, H. Wolf, E. Prohaska, *Anal. Chem.* **74**, 4488 (2002).
15. H.A. Ho, M. Boissinot, M.G. Bergeron, G. Corbeil, K. Doré, D. Boudreau, M. Leclerc, *Angew. Chem. Int. Ed.* **41**, 1548 (2002).
16. M. Leclerc, *Adv. Mater.* **11**, 1491 (1999).
17. K. Faïd, M. Leclerc, *J. Am. Chem. Soc.* **120**, 5274 (1998).
18. S. Bernier, S. Garreau, M. Béra-Abérem, C. Gravel, M. Leclerc, *J. Am. Chem. Soc,* **124**, 12463 (2002).

19. H.A. Ho, M. Leclerc, *J. Am. Chem. Soc.* **125**, 4412 (2003).
20. S. Basu, A.A. Szewczak, M. Cocco, S.A. Strobel. *J. Am. Chem. Soc.* **122**, 3240 (2000).
21. H. Ueyama, M. Takagi, S. Takenaka, *J. Am. Chem. Soc.* **124**, 14286 (2002).
22. L.C. Bock, L.C. Griffin, J.A. Latham, E.H. Vermaas, J.J. Toole, *Nature* **355**, 564 (1992).
23. K.Y. Wang, S. McCurdy, R.G. Shea, S. Swaminathan, P.H. Bolton, *Biochemistry,* **32**, 1899 (1993).
24. K. Padmanabhan, K.P. Padmanablan, J.D. Ferrara, J.E. Sadler, A. Tulinsky, *J. Biol. Chem,* **268**, 17651 (1993).
25. D.T. McQuade, A.E. Pullen, T.M. Swager, *Chem. Rev.* **100**, 2537 (2000).
26. L. Chen, D.W. McBranch, H.L. Wang, R. Hegelson, F. Wudl, D.G. Whitten, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 12287 (1999).
27. C. Fan, K.W. Plaxco, A.J. Heeger, *J. Am. Chem. Soc.* **124**, 5642 (2002).
28. B.S. Gaylord, A.J. Heeger, G.C. Bazan, *J. Am. Chem. Soc.* **125**, 896 (2003).
29. C. Fan, T. Hirasa, K.W. Plaxco, A.J. Heeger, *Langmuir* **19**, 3554 (2003).
30. K.P.R. Nilsson, M.R. Andersson, O. Inganäs, *J. Phys. Condens. Matter* **14**, 10011 (2002).
31. M. Michaud, E. Jourdan, C. Ravelet, A. Villet, A. Ravel, C. Grosset, E. Peyrin, *Anal. Chem.* **76**, 1015 (2004).